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Pre-, Peri-, and Postoperative Chemotherapy for Breast Cancer: Is One Better Than the Other?

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The purpose of the present study was to determine the relative efficacy of pre-, peri-, and postoperative chemotherapy in the prevention of breast cancer relapse and prolongation of host survival. The studies were performed using an experimental mouse breast cancer model. TA3Ha mouse mammary adenocarcinoma was transplanted into the mammary fat pad of syngeneic mice to obtain tumors in their natural organ. The tumors were surgically excised with a "curative" intent. A single treatment with 10 mg/kg doxorubicin was given intravenously pre-, peri-, or postoperatively. Among 74 mice whose tumors were resected but no doxorubicin was given, local recurrence, axillary metastasis, and lung metastasis were seen in 43%, 37%, and 16% of the mice, respectively. Seventeen (23%) mice had no evidence of disease. Doxorubicin given 4 days preoperatively reduced the rate of growth of primary tumor. Local recurrence was reduced in these mice by 30% and metastasis to the axillary lymph nodes and lung was completely prevented. Disease-free survival was increased to 70% ($P < 0.01$). Similar beneficial effects were obtained when chemotherapy was administered 2 days prior to surgery. The peri-operative chemotherapy group showed 8% (2/26) local recurrence, 4% axillary metastasis, and 0% lung metastasis. Proportion of mice without any evidence of disease increased to 92% ($P < 0.00001$). Chemotherapy given 4 days postoperatively resulted in 63% (10/16) local recurrence, 38% axillary metastasis, and 6.3% lung metastasis. Only 38% of the mice were disease-free. Thus in the model studied, perioperative chemotherapy offers the best chance for reduced recurrence and for improved disease-free survival.

KEY WORDS: **breast cancer, local recurrence, metastasis, timing of chemotherapy**

INTRODUCTION

Clinical experience over the past 40 years has shown that adjuvant chemotherapy to cancer patients yields superior clinical results compared to surgical treatment alone. In a recent analysis of 133 randomized trials involving 18,403 breast cancer patients, chemotherapy was found to reduce the risk of relapse by 21% and mortality by 11% [1]. The question, then, is not whether chemotherapy offers any benefit or not but how to further improve these benefits. In addition to the choice of drugs, the dosage, route of administration, and so forth, an important variable that can conceivably improve the treatment outcome is the timing of chemotherapy in relation to surgery. As highlighted eloquently by Fisher and Mamounas [2] in a recent editorial, no randomized clinical study or experimental animal study has evaluated the relative merits of pre-, peri-, and postoperative chemotherapy under

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similar conditions. Thus the means to maximize the benefits of chemotherapy based on the sequence and schedule are yet to be determined.

In the 1960s, chemotherapy was given perioperatively with an idea to eradicate cancer cells that may be shed or spread during surgery. In the early 1970s, it became customary to use chemotherapy weeks after surgery with the focus on eradicating the cells that are already disseminated [2]. Recently, with greater emphasis on reducing the extent of surgery and conserving the breast, there is a resurgence of interest to use chemotherapy prior to surgery. The rationale is that preoperative chemotherapy reduces the tumor size and permits less radical surgery. It is, however, not yet known whether the shrinkage of primary tumor prior to surgery translates into reduced relapse and mortality. The on-going clinical studies by Powles et al. [3] and the NSABP B-18 trial [2], which compare the efficacy of pre- and postoperative chemotherapy, are hoped to provide important information in this regard.

Surprisingly, little data are available from experimental animal studies on the relative merits of pre-, peri-, and postoperative chemotherapy. In an early study, Corbett et al. [4] utilized a mouse mammary carcinoma model to evaluate preoperative and postoperative doxorubicin (DOX) given as a single treatment. Surgical excision of subcutaneously transplanted tumors without chemotherapy yielded 17% cures (3/18) compared to 13% (2/15) in 3-day postoperative chemotherapy group and 67% (12/18) in 3 day preoperative chemotherapy group. These results demonstrate that preoperative treatment is better than no chemotherapy or postoperative chemotherapy. Unfortunately, no follow-up study is available on this important observation.

The present studies were undertaken to determine the optimal timing of chemotherapy in relation to surgical removal of the primary breast cancer in an experimental model. In this model, TA3Ha mouse mammary adenocarcinoma is implanted into the mammary fat pad of syngeneic mice so that the primary tumor is in its natural organ. Tumors at this site grow progressively and metastasize to the axillary lymph nodes and then to the lungs and other internal organs. This pattern is reminiscent of what happens in untreated breast cancer patients. By surgical excision of the tumor, some but not all mice can be cured. Those mice that fail surgical treatment develop local recurrence and/or distant metastases closely mimicking the situation encountered in breast cancer patients. Using this model, we examined the effects of single dose DOX given before, during or after surgical excision of the tumor.

MATERIALS AND METHODS

Mice

Strain A female mice were obtained from the National Cancer Institute, Frederick Cancer Research Facility (Frederick, MD). Mice were acclimatized to the Evanston Hospital animal care facility for 3 or more days prior to use. Mice were 8–10 weeks of age and weighed an average of 20g at the beginning of each experiment. All animal experiments were reviewed and approved by the Evanston Hospital Institutional Animal Care and Usage Committee.

Reagents

Hanks' Balanced Salt Solution (HBSS), L-15 medium, and fetal bovine serum were purchased from the Central Facilities, Northwestern University Cancer Center (Chicago, IL). Injectable doxorubicin (DOX) was from Ben Venue Laboratories (Bedford, OH). Nembutal (pentobarbital) was purchased from the Abbott Laboratories (North Chicago, IL).

Tumor Cells

Spontaneous mammary adenocarcinoma line TA3Ha [5] and syngeneic Strain A mice were used. TA3Ha is routinely maintained in our laboratory as ascites tumor in vivo. When these cells are inoculated into the mammary fat pad or subcutaneous tissue of mice, they yield solid tumors [6]. For each experiment, ascites cells were freshly harvested, washed in Ca^{++} and Mg^{++} free HBSS, and resuspended in L-15 medium supplemented with 10% fetal bovine serum. Each mouse was injected with 10^5 cells in 0.05 mL into the mammary fat pad.

Tumor Measurements

Tumors growing at the mammary fat pad were measured in cm at 3 orthogonal diameters 7 days after tumor injection. From these measurements, geometric mean diameter (GMD) of each tumor was calculated as described earlier [7]. The tumors that were not surgically treated were monitored for their rate of growth from day 7 to day 15. The effects of 10 mg/kg DOX on tumor growth rate were similarly monitored. The tumor growth rate was calculated based on the relative tumor size (RTS). RTS is the ratio of the GMD on the first day of measurement and the GMD on the day in question [7]. RTS of each tumor was plotted as a function of time (days) and the slope of linear regression curve was calculated. The mean and standard deviations of the slopes from each group were calculated.

Administration of Chemotherapy

Doxorubicin (10 mg/kg) was administered intravenously (IV) via the tail vein in a volume of 0.1 mL. Mice in the perioperative chemotherapy group received DOX immediately after surgery. Those that were assigned to the postoperative treatment were given the drug 2 or 4 days after surgery. Preoperative treatment was given 2 or 4 days prior to surgery. No evidence of toxicity or wound healing complication was seen.

TABLE I. Effects of Pre-, Peri-, and Postoperative Doxorubicin on Relapse of TA3Ha Mammary Carcinoma†

Group	Surgery	Doxorubicin mg/kg	Doxorubicin Schedule	No. mice	Local failure	Axillary metastases	Lung metastases	Free of disease d23*
1.	No	0	NA	9	9 (100)	4 (44)	3 (33)	0 (0)
2.	No	10	7 days postinjection	8	8 (100)	2 (25)	0 (0)	0 (0)
3.	Yes	0	NA	74	32 (43)	27 (37)	12 (16)	17 (23)
4.	Yes	10	4 days preoperative	10	3 (30)	0 (0)	0 (0)	7 (70)
5.	Yes	10	2 days preoperative	11	3 (28)	0 (0)	0 (0)	8 (73)
6.	Yes	10	Perioperative	26	2 (8)	1 (4)	0 (0)	24 (92)
7.	Yes	10	2 days postoperative	8	1 (13)	2 (25)	1 (13)	6 (75)
8.	Yes	10	4 days postoperative	16	10 (63)	6 (38)	1 (6)	6 (38)
9.	Yes	5	Perioperative	19	8 (42)	3 (16)	0 (0)	9 (47)
10.	Yes	2.5	Perioperative	8	5 (63)	1 (13)	0 (0)	3 (38)

†Numbers in parenthesis = percentage.
*Significance of the difference in disease-free survival: $P < 0.000002$ group 3 vs. group 6; $P < 0.007$ group 3 vs. groups 4, 5, or 7; $P < 0.0006$ group 6 vs. group 8.
NA = not applicable.

Tumor Excision

Immediately prior to surgery, the mice were anesthetized by intraperitoneal (IP) injection of 70 mg/kg pentobarbital. The skin over the tumor was prepared by applying 70% ethanol and betadine. The entire tumor (visible under a 4× magnifying lens) was removed with a "curative" intent. The skin wound was closed using 5-0 polyglactin suture and PS3 needle. Mice were transferred to their modules after recovering from anesthesia.

Data Collection

Postsurgical mice were monitored for 3 weeks after removal of their primary tumor (a time when all relapses and some deaths occur). The surviving mice were euthanized by i.p. injection of 150–200 mg/kg Nembutal. All experimental mice were subjected to a complete autopsy examination. The sites and numbers of recurrence were noted from the autopsy examination. The number of mice that were free of macroscopic disease at the time of euthanasia was noted. Statistical analysis was performed by Chi-squared test.

RESULTS

The results are summarized in Table I. Every mouse inoculated with 10^5 TA3Ha cells into the mammary fat pad developed a local solid tumor. By day 7, the average GMD was 0.62 ± 0.05 cm. These tumors increased in size at a rate of 0.159 ± 0.06 cm/day ($r = 0.979$) during day 7 through day 15. Tumors in all these mice invaded the peritoneum and formed hemorrhagic ascites tumor. Ipsilateral axillary metastasis and lung metastasis were seen in 44% and 33% of the mice, respectively. The survival period of these mice was $\sim 17 \pm 4$ days. When the mice were given 10 mg/kg DOX on day 7 of tumor injection, the tumors increased in size at a rate of 0.087 ± 0.049 cm/day ($r = 0.972$). As in the untreated controls, the treated tumors also invaded the peritoneum and formed ascites tumor. Two of eight mice had ipsilateral axillary metastasis, and none had lung metastasis. The tumor was 100% lethal with a host survival period of approximately 21 days.

Effects of Surgery

The effects of "curative" surgical excision of tumors were studied in 74 mice. Thirty-two (43%) mice had local recurrence (i.e., tumor in the surgical scar), 37% had ipsilateral axillary metastasis, and 16% had lung metastasis. Seventeen (23%) mice were disease-free.

Effects of Preoperative Chemotherapy

Two groups of mice were assigned to preoperative chemotherapy. They were treated 4 or 2 days prior to surgery. The average GMDs of tumors on day 7 (day of surgery) in these groups were 0.37 ± 0.06 and 0.50 ± 0.08 cm, respectively. Thus the preoperative chemotherapy mice had smaller tumors compared to the untreated mice (0.62 ± 0.05 cm) at time of surgery. In both these groups, there was ~30% reduction in local recurrence. Metastasis to the axillary nodes or the lungs was completely prevented. Proportion of disease-free survivors increased from 23% to ≥70% ($P < 0.01$).

Effects of Perioperative Chemotherapy

Twenty-six mice were given 10 mg/kg DOX immediately after removal of the primary cancer. Among these mice, 8% had local recurrence, 4% had ipsilateral axillary metastasis, and none (0%) had lung metastasis. Twenty-four (92%) mice were disease-free. The proportion of disease free survivors is significantly ($P < 0.00001$) greater compared to the surgery alone group. Among 19 mice that received 5 mg/kg DOX, 42% had local recurrence, 16% had ipsilateral axillary metastasis, and

none (0%) had lung metastasis. Nine (47%) mice were disease-free. Among eight mice treated with 2.5 mg/kg, 63% had local relapse, 13% had ipsilateral axillary metastasis, and 63% had lung metastasis. Three (38%) mice were disease-free. Disease-free survival was directly related to the dose of DOX (correlation coefficient = 0.986).

Effects of Postoperative Chemotherapy

Among eight mice given 10 mg/kg DOX 2 days after surgery, 13% had local recurrence, 25% had ipsilateral axillary metastasis, and none had lung metastasis. Six (75%) mice were disease-free ($P < 0.01$). Among 16 mice given 10 mg/kg DOX 4 days after surgical excision of the primary tumor, 63% had local recurrence, 38% had ipsilateral axillary metastasis, and 6% had lung metastasis. Six (37.5%) mice were disease-free. The proportion of disease-free survivors is significantly ($P < 0.0006$) less compared to the perioperative group. These effects are equivalent to those obtained with 2.5 mg/kg doxorubicin administered perioperatively and also to those in the surgery alone group. Thus a delay of 4 days in administration of DOX completely obviated the beneficial effects of chemotherapy.

DISCUSSION

It has clearly been shown that a combination of chemotherapy and surgery yields superior clinical results compared to surgery alone [8–13]. Data collected by the Early Breast Cancer Trialists' Collaborative Group from 133 randomized trials involving 18,403 women with breast cancer revealed that chemotherapy reduces the risk of recurrence by 21% and mortality by 11% [1]. Thus whether combination of surgery and chemotherapy offers any benefit over surgery alone is not an issue any longer. But the concern is further improvement of chemotherapy benefits. A dictum for adjuvant chemotherapy is that many cancers are curable if treated at a time when the tumor burden is the least [14]. Thus it is to be expected that chemotherapy instituted intraoperatively or immediate postoperatively provides the best results. Furthermore, poor clinical results may be predicted if a remnant tumor is allowed time for recovery prior to chemotherapy.

This prediction is substantiated by the results from an experimental mouse breast cancer study of Corbett et al. [4]. They showed that Adriamycin given 3 days preoperatively cured 67% of the mice, whereas the same chemotherapeutic agent produced 13% cures when given 3 days postoperatively. No comparison to perioperative treatment was made. In our studies, perioperative chemotherapy accomplished a 92% cure rate. This cure rate is clearly superior to 75% cure rate with 2-day postoperative treatment and 37% ($P < 0.0006$) cure rate with 4-day postoperative treatment. The results are impressive considering that a single treatment of a single agent was utilized. These results are strikingly similar to the clinical experience of Hines [15] with breast cancer patients treated perioperatively with a single cycle of nitrogen mustard. In his series, only one of 39 patients developed recurrence over a 30-year follow-up period.

The reasons why postoperative treatments are inferior to perioperative treatment are not clear. One possible explanation involves growth stimulation of the residual cancer following surgery. Fisher and colleagues [16,17] have demonstrated that surgical excision of a tumor results in the production and secretion of growth factors into the circulation. These growth factors stimulate the proliferation of any residual tumor. When chemotherapy is given pre- or perioperatively, the production of growth factors as well as the stimulation of remnant tumor are reduced. These findings imply that the tumor burden during the postoperative period may increase sufficiently to overwhelm the anticancer agent. However, this is unlikely to be the sole mechanism since preoperative treatment that encounters the primary tumor is still effective. One may speculate that in addition to the growth stimulation, the physiological events following surgical wounding may protect the tumor cells against chemotherapy. However, this hypothesis still needs to be tested.

The compelling reason for the use of preoperative chemotherapy is to reduce the tumor bulk (down stage the cancer) so that surgery can be less radical. By avoiding radical procedures, breast conservation surgery may be accomplished. Our results demonstrate that the tumors treated with DOX preoperatively are smaller at time of surgery compared to those not previously treated. This clearly lends support to the current thoughts that preoperative chemotherapy may have a role in reducing the extent of surgery. However, there is little clinical data to predict whether reduction of tumor size for purposes of reducing the extent of surgery also translates into reduced recurrence and mortality. The data we have presented and those by Corbett et al. [4] strongly indicate that preoperative treatment indeed offers significant benefits. These are reduction of local recurrence and distant metastasis, as well as improvement in disease-free survival. However, for those tumors that are less advanced and are amenable to surgery, a perioperative treatment is likely to offer greater benefits.

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